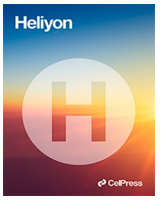
Heliyon 10 (2024) e33304

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Systematic pan-cancer analysis identified RASSF1 as an immunological and prognostic biomarker and validated in lung cancer

Yibing Bai [a,b,1], Yuanyong Wang [c,1], Jiapei Qin [a,b,1], Ting Wang [b,d,1], Xin Zhou [a,b], Zhiqiang Ma [b], An Wang [a,b], Wenyu Yang [b,d], Jinliang Wang [a,b,**], Jinfeng Li [e,***], Yi Hu [a,b,*]

[a] Medical School of Chinese PLA, Beijing, China
[b] Department of Oncology, The First Medical Center of PLA General Hospital, Beijing, China
[c] Department of Thoracic Surgery, Tangdu Hospital, Air Force Medical University, Xi'an, China
[d] School of Medicine, Nankai University, Tianjin, China
[e] Institute of Oncology, Senior Department of Oncology, The Fifth Medical Center of PLA General Hospital, Beijing, China

ARTICLE INFO

Keywords:
RASSF1
Pan-cancer
Prognosis
Immunity
Lung cancer

ABSTRACT

Background: Ras association domain family member 1 (RASSF1) encodes the RASSF1A protein, serving as a scaffold protein situated at the intersection of a complex signalling network.
Aims: To evaluate the immunological and prognostic significance of RASSF1 expression in various types of human cancers, with a specific focus on lung cancer.
Methods: Differential expression analysis of RASSF1 was conducted based on data from The Cancer Genome Atlas, Genotype-Tissue Expression, and Cancer Cell Line Encyclopaedia databases. Prognostic analysis was performed using the Cox regression test and Kaplan-Meier test. Spearman's test was utilized for correlation analysis. Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) gene sets were employed to enrich the associated signaling pathways. Immunohistochemical staining and quantitative real-time PCR were employed to detect protein and mRNA expression levels, respectively.
Results: RASSF1 expression was significantly lower in tumour tissues than in normal tissues in most cancers, and Cox regression analysis demonstrated a significant correlation between RASSF1 expression and the prognosis of over 12 types of cancer. Specifically, high RASSF1 expression was associated with poor OS in nine cancer types, including GBMLGG (HR = 4.98, P = 1.2e-31), LGG (HR = 3.72, P = 2.5e-10), and LAML (HR = 1.48, P = 2.4e-3). Further analysis showed that RASSF1 expression was significantly correlated with immune checkpoint- and immune-related genes. Moreover, RASSF1 expression is involved in tumour microenvironment (TME), RNA modification, genomic heterogeneity, and tumour stemness. GO and KEGG analyses showed that RASSF1 was closely related to tumour immune-related pathways. Finally, RASSF1A was moderately correlated with PD-L1 (R = 0.556), and RASSF1A overexpression significantly

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: wangjinliang301@126.com (J. Wang), lijinfeng148@163.com (J. Li), huyi301zlxb@sina.com (Y. Hu).
[1] YBB, YYW, JPQ and TW served as co-first authors.

https://doi.org/10.1016/j.heliyon.2024.e33304
Received 26 December 2023; Received in revised form 15 June 2024; Accepted 18 June 2024
Available online 21 June 2024

affected the expression of several genes involved in the Th17 cell differentiation signalling pathway in lung cancer.
Conclusions: RASSF1 was differentially expressed in 29 human cancers and played a critical role in tumour immunity. Thus, RASSF1 has the potential to be used as a prognostic marker and reference for achieving more precise immunotherapy, particularly in lung cancer.

1. Introduction

Human health is facing an escalating threat from cancer, which stands as one of the foremost causes of death globally [1]. Despite the exploration of diverse approaches to cancer treatment, the prognosis for patients with advanced tumours remains uncertain. In recent times, remarkable strides have been achieved in human immunotherapy. It can be asserted that immunotherapy is revolutionizing antitumour therapy like never before [2,3]. Nevertheless, a significant proportion of patients fail to derive benefits from immunotherapy. Hence, there is a pressing need to identify novel immune-related targets and prognostic biomarkers.

Ras association domain family member 1 (RASSF1), located on chromosome 3p21.31, exhibits expression of eight major transcript variants Among these variants, the major and ubiquitous ones are RASSF1A and RASSF1C. Additional subtypes include RASSF1B, RASSF1D to H [4]. Serving as a prototypical tumour suppressor gene, RASSF1A is frequently inactivated across more than 40 human malignant tumours [5]. Hypermethylation of the RASSF1A promoter region stands as a pivotal early event in cancer development. Consequently, the detection of RASSF1A methylation holds promise for diagnosing various cancers at an early stage. Studies have demonstrated that the methylation levels of the RASSF1A promoter region in pathological tissues [6] and alveolar lavage fluid [7] can aid in the diagnosis of lung cancer. In breast cancer, the sensitivity, diagnostic odds ratio (DOR) and area under the curve (AUC) of serum RASSF1A methylation were 0.55, 22.0 and 0.86, respectively [8]. Moreover, the sensitivity, specificity, and accuracy of detecting RASSF1A, APC, and FOXA1 promoter methylation in circulating cell-free DNA (ccfDNA) for the diagnosis of breast cancer are greater than 70 % [9]. In colorectal cancer, RASSF1A methylation ranges from 12 % to 81 %. A meta-analysis revealed that RASSF1A promoter methylation was associated with a significantly increased risk of colorectal cancer (odds ratio [OR]: 6.53, 95 % confidence interval (CI): 3.88–11.01, $P < 0.001$) and poor overall survival (hazard ratio: 2.85, 95 % CI: 1.88–4.31, $P < 0.001$) [10,11]. Kim et al. found that the detection of RASSF1A methylation in urine has good diagnostic value in patients with HCC and can be used as a potential non-invasive screening method for HCC [12]. Khoshfetrat et al. found that early diagnosis of thyroid cancer could be achieved by detecting RASSF1A and SLC5A8 promoter methylation levels in the blood [13]. In addition to its diagnostic value, RASSF1A methylation has a prognostic value in several tumours. Patients with non-small cell lung cancer (NSCLC) and RASSF1A hypermethylation have poor prognosis [14]. RASSF1A hypermethylation is also associated with high-grade advanced cervical cancer [15]. In addition, RASSF1A hypermethylation is associated with the TNM stage of gastric cancer, and the frequency of hypermethylated RASSF1A is significantly higher in stage III and IV patients than in stage I and II [16].

There is growing evidence that RASSF1A, a scaffold protein involved in cell signalling, targets key regulators of cell homeostasis such as Ras, MST2/Hippo, p53, and death receptor pathways [17]. According to an article published in CELL, the Hippo signalling pathway can inhibit tumour initiation and suppress immunogenicity. Loss of the Hippo pathway LATS1/2 can promote the growth of tumour cells, and LATS1/2 deficiency can enhance the efficacy of tumour vaccines, improve the immunogenicity of tumours, and lead to tumour destruction by enhancing anti-tumour immune responses [18]. As a scaffold protein of the Hippo signalling pathway, RASSF1A can bind to MST1/2 and promote its activation, thereby activating the Hippo signalling pathway. LATS1/2 is the main effector of MST1/2, which inactivates the transcriptional activity of the YAP/TAZ protein [19]. These results suggested that RASSF1A may also play a role in tumour immunosuppression. In addition, RASSF1A inhibition promotes B cell transformation, which is an important process in the immune response. EBV latent antigen 3C (EBNA3C) directly interacts with RASSF1A and induces RASSF1A degradation through a ubiquitin-proteasome-dependent pathway, thereby promoting B cell transformation [20]. In summary, a growing amount of evidence suggests that RASSF1A can not only be used as a marker for the early screening and diagnosis of tumours and prognosis but may also play an important role in tumour immunity. While the exploration of RASSF1C is limited, earlier studies have shown that RASSF1C induces cell cycle arrest in cancer cell lines, indicating that it can act like RASSF1A in suppressing tumours [21]. However, other findings support a potential carcinogenic role of RASSF1C. In contrast to RASSF1A, RASSF1C overexpression can inhibit betaTrCP to promote the accumulation and transcriptional activation of β-catenin [22]. Additionally, RASSF1C promotes the proliferation of lung cancer cells [23] and drives mesenchymal-amoeboid transformation and stem cell properties of breast cancer cells [24].

Increasing evidence has shown that RASSF1 plays an important role in the occurrence and development of various tumours, including liver, esophageal, and non-small-cell lung cancers. Moreover, RASSF1A is not only involved in early tumorigenesis but also in immune regulation. However, mechanisms underlying the effects of RASSF1 on tumorigenesis and immunity remain unclear. Moreover, most studies on the role of RASSF1 in tumours have been limited to specific types of cancer. To date, there have been no pan-cancer studies investigating the association between RASSF1 and various cancers. To better understand the key role of RASSF1 in tumour development and immunity, we comprehensively analysed the prognostic value and immune function of RASSF1 in different tumour types using multiple databases.

First, we used the Genotype Tissue Expression (GTEx), Encyclopaedia of Cancer Cell Lines (CCLE), and The Cancer Genome Atlas Program (TCGA) databases to determine the RASSF1 expression levels in common tumours and their relationship with prognosis. In addition, we explored the correlation between RASSF1 expression levels and immune checkpoints, immunomodulatory genes, and the

tumour microenvironment (TME) in multiple malignancies. Furthermore, the role of RASSF1 in RNA modification, genomic heterogeneity, and tumour stemness has been analysed in a variety of cancers. We mined the potential genes and signalling pathways associated with RASSF1 using protein interaction (PPI) network analysis, as well as Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) gene set enrichment analyses in multiple malignancies. According to the above analysis, RASSF1 expression was significantly lower in NSCLC tissues than in normal lung tissues, and RASSF1 expression was significantly correlated with PD-L1 expression in NSCLC. Therefore, we verified that RASSF1A was moderately correlated with PD-L1, and that RASSF1A overexpression significantly affected the expression of several genes involved in the Th17 cell differentiation signaling pathway in lung cancer. Taken together, our pan-cancer analysis provides insights into the therapeutic and prognostic roles of RASSF1 in common cancers, and sheds new light on its role of RASSF1 in tumour immunotherapy.

2. Methods

2.1. Cell culture and transfection

A549 and HEK-293T cells were obtained from the American Type Culture Collection (ATCC, VA, USA), and cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 1 % penicillin/streptomycin (Solarbio) and 10 % foetal bovine serum (FBS; Vivacell). RASSF1A and empty vector lentiviral plasmids were obtained from GeneChem (Shanghai, China). Lentiviral infection was performed in A549 cells according to the protocol described in the GeneChem Recombinant Lentivirus Operation Manual.

2.2. Data acquisition and differential expression analysis

Tumour cell line data for RASSF1 expression were downloaded from the CCLE (https://portals.broadinstitute.org/ccle/) database,

Table 1
Abbreviations of the cancers.

Cohort	Tumor Name	Abbreviations
TCGA	Adrenocortical carcinoma	ACC
TCGA	Bladder urothelial carcinoma	BLCA
TCGA	Breast invasive carcinoma	BRCA
TCGA	Cervical squamous cell carcinoma and endocervical adenocarcinoma	CESC
TCGA	Cholangiocarcinoma	CHOL
TCGA	Colon adenocarcinoma	COAD
TCGA	Colon adenocarcinoma/Rectum adenocarcinoma Esophageal carcinoma	COADREAD
TCGA	Lymphoid neoplasm diffuse large B-cell lymphoma	DLBC
TCGA	Esophageal carcinoma	ESCA
TCGA	Glioblastoma multiforme	GBM
TCGA	Glioblastoma multiforme low-grade glioma	GBMLGG
TCGA	Head and neck squamous cell carcinoma	HNSC
TCGA	Kidney chromophobe	KICH
TCGA	Pan-kidney cohort (KICH + KIRC + KIRP)	KIPAN
TCGA	Kidney renal clear cell carcinoma	KIRC
TCGA	Kidney renal papillary cell carcinoma	KIRP
TCGA	Acute myeloid leukemia	LAML
TCGA	Brain lower grade glioma	LGG
TCGA	Liver hepatocellular carcinoma	LIHC
TCGA	Lung adenocarcinoma	LUAD
TCGA	Lung squamous cell carcinoma	LUSC
TCGA	Mesothelioma	MESO
TCGA	Ovarian serous cystadenocarcinoma	OV
TCGA	Pancreatic adenocarcinoma	PAAD
TCGA	Pheochromocytoma and Paraganglioma	PCPG
TCGA	Prostate adenocarcinoma	PRAD
TCGA	Rectum adenocarcinoma	READ
TCGA	Sarcoma	SARC
TCGA	Stomach adenocarcinoma	STAD
TCGA	Skin cutaneous melanoma	SKCM
TCGA	Stomach and esophageal carcinoma	STES
TCGA	Testicular germ cell tumours	TGCT
TCGA	Thyroid carcinoma	THCA
TCGA	Thymoma	THYM
TCGA	Uterine corpus endometrial carcinoma	UCEC
TCGA	Uterine carcinosarcoma	UCS
TCGA	Uveal melanoma	UVM
TARGET	Osteosarcoma	OS
TARGET	Acute lymphoblastic leukemia	ALL
TARGET	Neuroblastoma	NB
TARGET	High-risk wilms tumor	WT

and the expression levels of RASSF1 in 21 tumour cell lines were analysed. RNA sequencing and related clinical data for normal and tumour tissues were collected from the GTEx (https://commonfund.nih.gov/GTEx/) and TCGA (https://portal.gdc.cancer.gov) databases, respectively. The R package was used to analyse and present the data. Statistical significance was set at $P < 0.05$. Difference in RASSF1 expression between normal and tumour tissues was analysed using the Wilcoxon rank-sum test.

2.3. Analysis of prognosis

TARGET follow-up data were obtained as a supplement, and samples with follow-up times of <30 days were excluded. We applied a $\log_2^{(x+0.001)}$ transformation to each expression value and excluded cancers with fewer than 10 samples in a single cancer (Table 1). Finally, for each sample, we collected expression data for overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free intervals (PFI). The prognostic role of RASSF1 in pan-cancer was analysed using the univariate Cox proportional hazards regression log-rank test and Kaplan-Meier (K–M) test.

2.4. RASSF1 expression correlated with immunomodulatory genes, immune checkpoints, and TME

Spearman's rank correlation coefficient was utilized to analyse the correlation between RASSF1 expression and immune checkpoints (both inhibitory and stimulatory) as well as immunomodulatory genes (including chemokines, chemokine receptors, and MHC). Stromal, immune, and ESTIMATE scores were computed for each patient in each cancer type based on gene expression, employing the R package ESTIMATE [25], followed by correlation analysis with RASSF1. Subsequently, using the deconvolutional mcpcounter method [26] of the R package IOBR [25], we reassessed the infiltration scores of immune cells for each patient in each cancer type based on gene expression, followed by correlation analysis with RASSF1.

2.5. Correlation between RASSF1 expression and RNA-modified genes

Based on Huang et al. [26] and 44 marker genes associated with three classes of RNA modifications (m1A, m5C, and m6A), we compared RASSF1 expression with that of RNA-modified genes using Spearman correlation.

2.6. Correlation between RASSF1 expression and genomic heterogeneity

The tumour mutation burden (TMB) and mutant-allele tumour heterogeneity (MATH) were calculated using the TMB and inferHeterogeneity functions of the maftools package (version 2.8.05). We acquired tumour neoantigen, microsatellite instability (MSI), and purity data from previous studies [27,28]. We downloaded the harmonised pan-cancer dataset from UCSC (https://xenabrowser.net/) database. After integrating the gene expression data, cancer species with fewer than three samples from a single cancer species were excluded. The tumours involved in each analysis are shown in Fig. 9, and the full tumour names are listed in Table 1. By integrating the gene expression data, the correlations between RASSF1 expression and TMB, tumour neoantigens, MSI, purity, and MATH were analysed.

2.7. Correlation between RASSF1 expression and tumour stemness

The DNA methylation-based stemness index (DNAsi) and mRNA expression-based stemness index (mRNAsi) were obtained from previous researches [29]. Cancer species with fewer than three samples from a single cancer species were excluded by integrating the gene expression data downloaded from the UCSC database (TCGA Pan-Cancer), cancer species with less than 3 samples in a single cancer species were excluded. The tumours involved in each analysis are shown in Fig. 10, and the full tumour names are listed in Table 1. We integrated the sample stemness index and gene expression data and analysed the correlation between RASSF1 expression and DNAsi and mRNAsi expression.

2.8. PPI, GO and KEGG enrichment analysis

PPI network analysis was conducted using STRING (https://cn.string-db.org/). R language was utilized to compute the correlation between RASSF1 and other genes in the pan-cancer dataset. GO and KEGG enrichment analyses were performed on correlated genes with a significance threshold of $P < 0.05$ and $|cor| > 0.3$. The ClusterProfiler package was employed for conducting the enrichment analysis, and the results were visualized using the ggplot2 package.

2.9. Clinical samples and immunohistochemistry (IHC) staining

IHC staining was performed on normal lung tissues and NSCLC samples from the Human Protein Atlas (HPA) (https://www.proteinatlas.org/). The study protocol was approved by the People's Liberation Army General Hospital and was used to collect immunohistochemical specimens. Written informed consent was obtained from all the patients in accordance with the Declaration of Helsinki. Goat serum was blocked to prevent nonspecific staining. Staining was performed using RASSF1A (1:100, 14-6888-82; Thermo Fisher Scientific) and PD-L1 (1:200, 13684S; CST).

2.10. Quantitative real-time polymerase chain reaction (qPCR)

Total RNA was extracted from A549 cells using the TRIzol reagent, and complementary DNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). qPCR was conducted using SYBR Green qPCR Master Mix and SYBR Premix Ex *Taq*II (Sellect, USA) to detect the target mRNA levels. The primer sequences used for qPCR are listed in Supplementary Table 1. Relative quantification was determined using the ΔΔCt method.

3. Results

3.1. Differential RASSF1 expression among tumour and normal tissues

The relative expression of RASSF1 in normal and tumour tissues was evaluated using three databases (GTEx, CCLE, and TCGA). Data from the GTEx dataset indicated that RASSF1 was commonly expressed in 31 normal tissues, with the highest expression levels in the nerve and spleen, and the lowest expression levels in the brain and liver (Fig. 1A). The relative RASSF1 expression in various tumour cell lines from the CCLE dataset is shown in Fig. 1B. Analysis of RASSF1 expression data in TCGA and GTEx matched tumour tissues and normal tissues showed that RASSF1 expression was significantly lower in tumour tissues of Breast invasive carcinoma (BRCA), Lung adenocarcinoma (LUAD), Lung squamous cell carcinoma (LUSC) and other 20 cancers than in normal tissues, while the

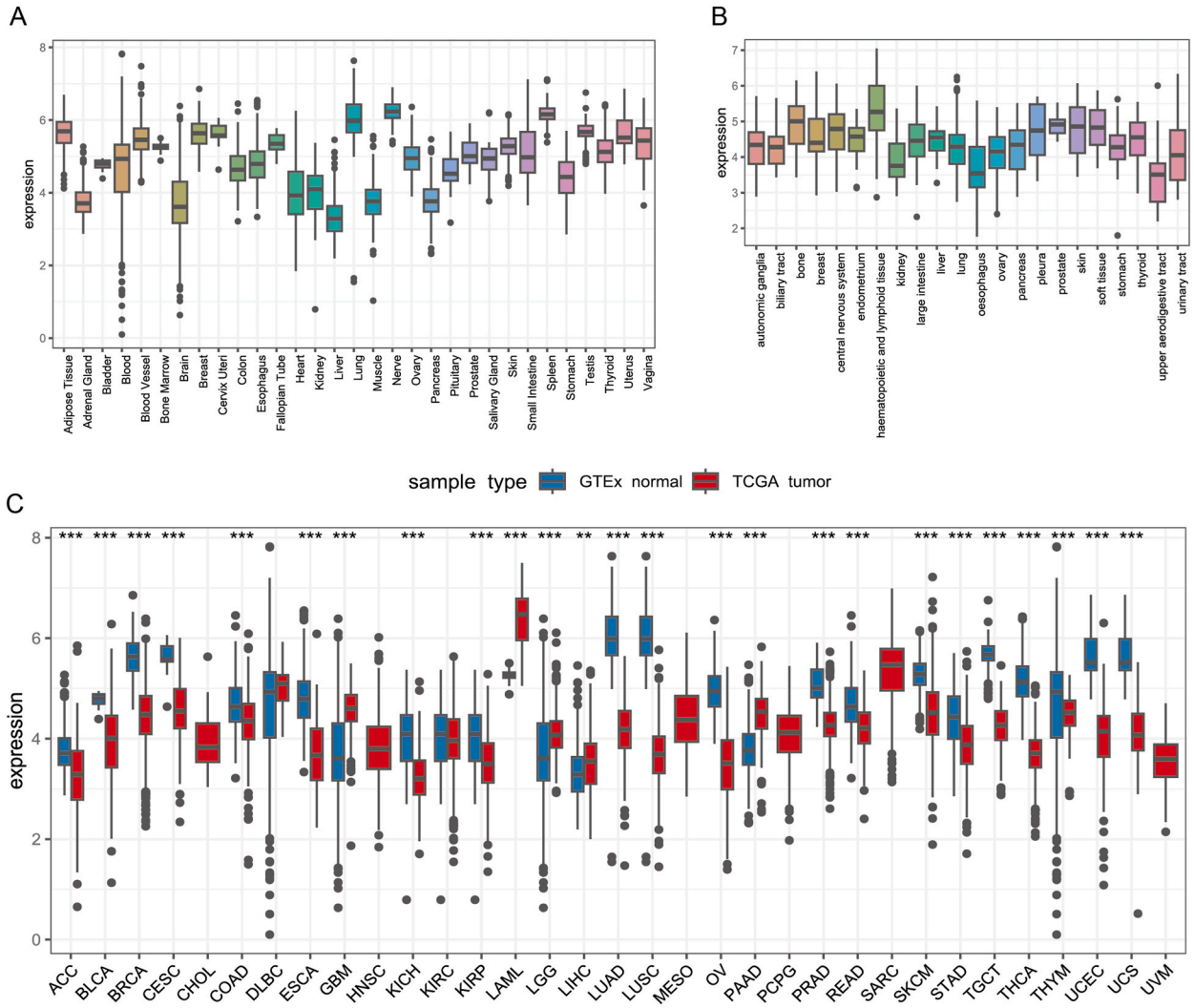


Fig. 1. Expression levels of RASSF1. (A) RASSF1 expression levels in normal tissues with data from the GTEx database. (B) RASSF1 expression levels in tumor cell lines with data from the CCLE database. (C) RASSF1 expression levels in tumor and normal tissues based on the consolidated data of the GTEx and TCGA databases. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. GTEx, Genotype-Tissue Expression; CCLE, Cancer Cell Line Encyclopaedia; TCGA, The Cancer Genome Atlas.

RASSF1 expression was higher in 9 cancers than in normal tissues, including Cholangiocarcinoma (CHOL), Glioblastoma multiforme (GBM), and Head and neck squamous cell carcinoma (HNSC) (Fig. 1C). According to these results, tumours generally express lower levels of RASSF1 than their corresponding normal tissues.

3.2. Prognostic value of RASSF1 in pan-cancer

To evaluate the role of RASSF1 in cancer prognosis, survival analyses were performed for each cancer type, including OS, DSS, DFI and PFI. Cox regression analysis demonstrated that high RASSF1 expression was associated with worse OS in nine types of cancer, including GBMLGG, LGG, and LAML, whereas in HNSC, TARGET-NB, and TARGET-ALL-R, low RASSF1 expression resulted in poor OS (Fig. 2A). Similar results were obtained using Kaplan-Meier survival analysis. RASSF1 expression was associated with poor OS in seven cancers, including ACC, BLCA, and GBM, but not in HNSC or THYM (Fig. 2B). Additionally, Cox analysis showed that high RASSF1 expression indicated poor DSS in eight cancers, including GBMLGG, LGG, and KIPAN; only in HNSC did low RASSF1 expression result in poor DSS (Fig. 3A). Kaplan–Meier survival analysis showed that high RASSF1 expression resulted in poor DSS in six tumour types, including ACC, GBM, and KICH (Fig. 3B).

Next, we analysed RASSF1 expression in various tumour types and its effect on PFI. Cox regression analysis showed that six tumour types (GBMLGG, LGG, PRAD, LIHC, ACC, and KICH) with high RASSF1 expression had poor prognoses, and only STES and STAD with low RASSF1 expression had poor prognoses (Fig. 4A). Kaplan-Meier survival analysis demonstrated that high RASSF1 expression in ACC, KICH, LGG, LIHC, and PRAD predicted poor PFI, whereas high RASSF1 expression in patients with STAD indicated better PFI (Fig. 4B). Finally, we evaluated the changes in the DFI at different RASSF1 expression levels. Cox regression analysis showed that high RASSF1 expression in PRAD, LIHC, and ACC was associated with poor DFI, whereas low RASSF1 expression in STES, STAD, and PCPG was associated with poor prognosis (Fig. 5A). The Kaplan–Meier survival curve showed that high RASSF1 expression was associated with poor DFI in the ACC and LIHC, whereas it was associated with better DFI in the PCPG and STAD (Fig. 5B).

3.3. RASSF1 is correlated with immune-related genes in pan-cancer

To explore the role of RASSF1 in tumour immunity, we first analysed the correlation between RASSF1 and immune checkpoints (including 24 immunosuppressants and 36 immune stimulators). There was a positive correlation between RASSF1 expression and immune checkpoint molecules in most cancer types, particularly TGCT, KIPAN, KIRP, GBMLGG, LGG, and BLCA. In addition, RASSF1 positively correlated with TGFB1, C10orf54, ADORA2A, TNFRSF14, ENTPD1, and CD28 in almost all cancers ($P < 0.05$) (Fig. 6A). Furthermore, the association of RASSF1 with 41 chemokines, 18 chemokine receptors, and 21 MHC molecules was analysed. Similarly,

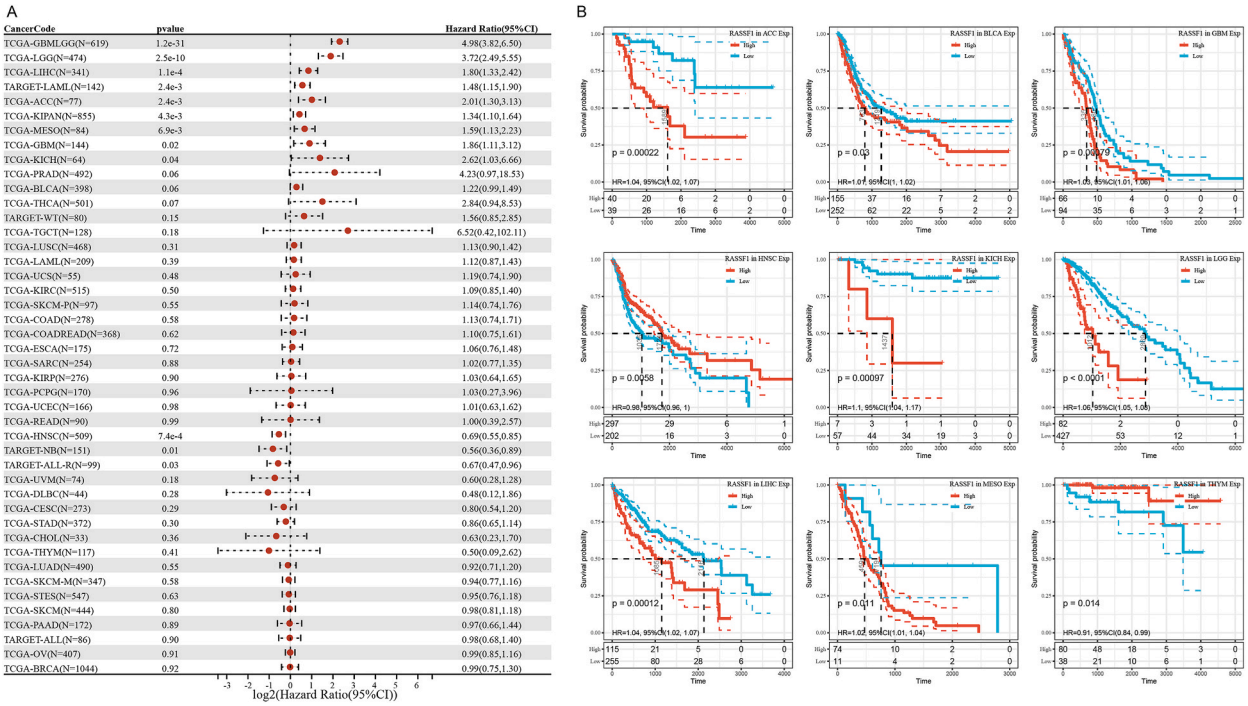


Fig. 2. Correlations between RASSF1 expression and OS. (A) Cox analysis of RASSF1 expression and OS in pancancer. (B) K–M analysis of RASSF1 expression and OS in ACC, BLCA, GBM, HNSC, KICH, LGG, LIHC, MESO, and THYM. OS, overall survival; K–M, Kaplan–Meier; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; MESO, mesothelioma; THYM, thymoma.

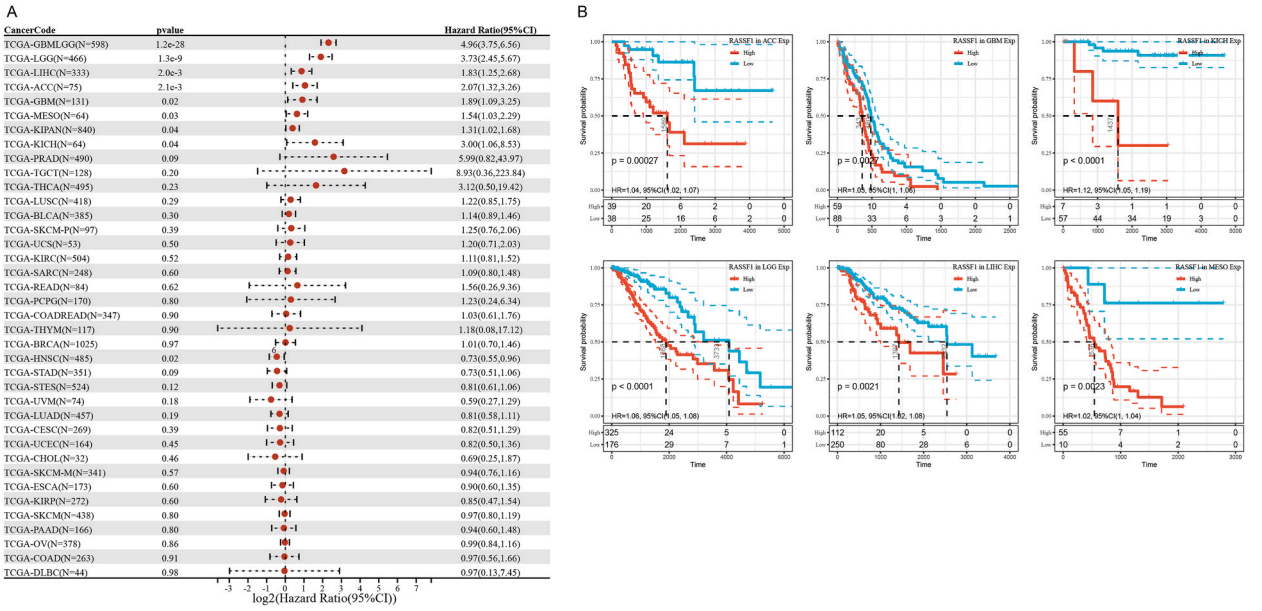


Fig. 3. Correlations between RASSF1 expression and DSS. (A) Cox analysis of RASSF1 expression and DSS in pancancer. (B) K–M analysis of RASSF1 expression and DSS in ACC, GBM, KICH, LGG, LIHC, and MESO. DSS, disease-specific survival; K–M, Kaplan–Meier; ACC, adrenocortical carcinoma; GBM, glioblastoma multiforme; KICH, kidney chromophobe; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; MESO, mesothelioma.

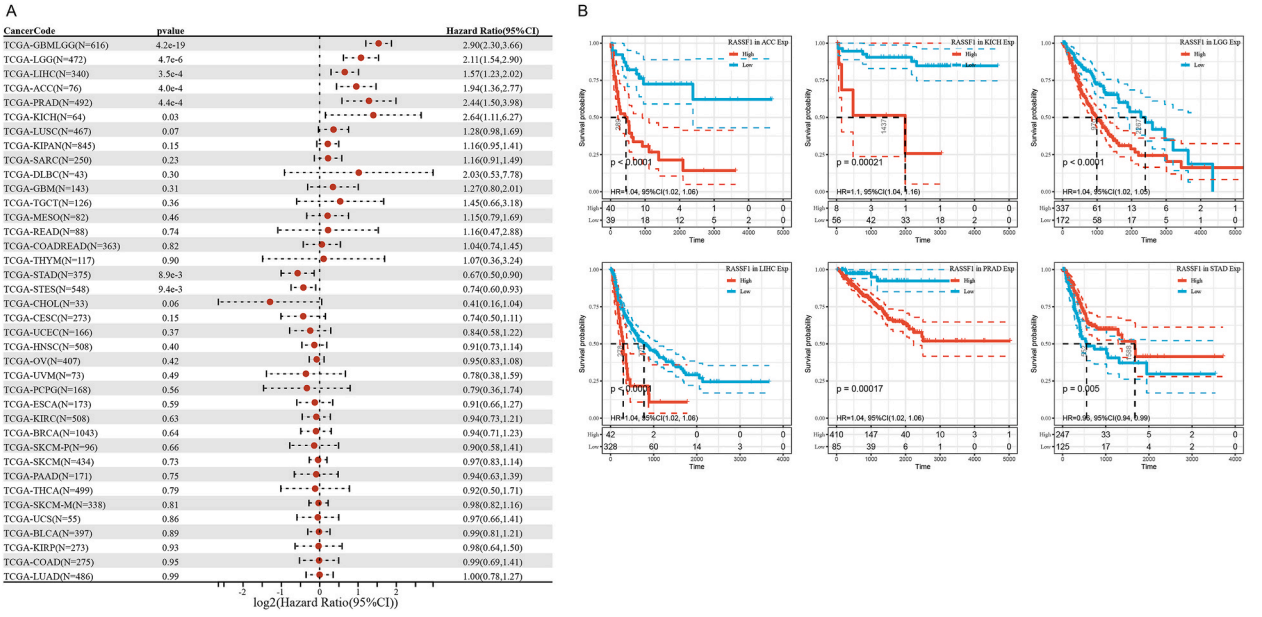


Fig. 4. Correlations between RASSF1 expression and PFI. (A) Cox analysis of RASSF1 expression with PFI in pancancer. (B–D) K–M analysis of RASSF1 expression and DFI in ACC, KICH, LGG, LIHC, PRAD, and STAD. PFI, progression-free interval; K–M, Kaplan–Meier; ACC, adrenocortical carcinoma; KICH, kidney chromophobe; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; PRAD, prostate adenocarcinoma; STAD, stomach adenocarcinoma.

RASSF1 was positively correlated with most immunomodulatory genes in most tumours, especially chemokine receptors and MHC molecules ($P < 0.05$). In KIPAN, KIRP, and LIHC, RASSF1 showed a significant positive correlation with almost all chemokines, chemokine receptors, and MHC ($P < 0.05$) (Fig. 6B).

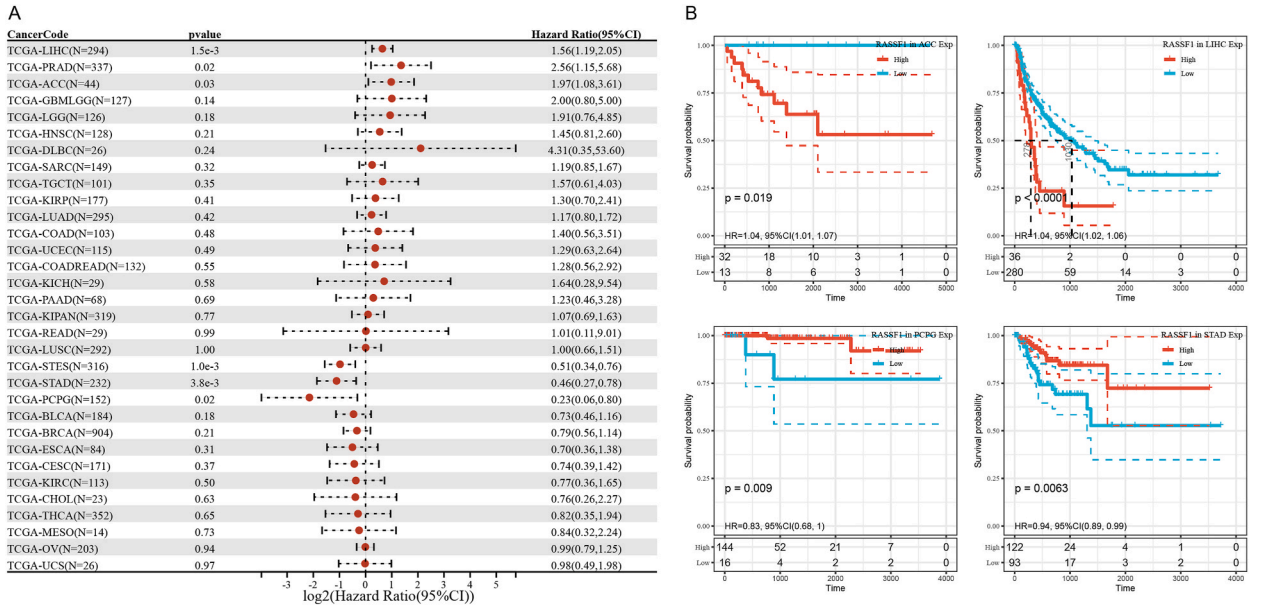


Fig. 5. Correlations between RASSF1 expression and DFI. (A) Cox analysis of RASSF1 expression with DFI in pancancer. (B–F) K–M analysis of RASSF1 expression and PFI in ACC, LIHC, PCPG, and STAD. DFI, disease-free interval; K–M, Kaplan–Meier; LGG, low-grade glioma; ACC, adrenocortical carcinoma; LIHC, liver hepatocellular carcinoma; PCPG, pheochromocytoma and paraganglioma; STAD, stomach adenocarcinoma.

3.4. *RASSF1 affects the tumour microenvironment and immune infiltration across tumours*

To explore the effect of RASSF1 on TME, we evaluated the correlation between RASSF1 expression and stromal, immune, and ESTIMATE scores using the ESTIMATE algorithm. RASSF1 expression significantly correlated with the stromal score in 28 tumours, including GBMLGG, KIPAN, and BLCA ($P < 0.05$). Among them, 27 correlations were positive and one was negative. RASSF1 expression was also significantly correlated with the immune score in 29 cancers, including GBMLGG, LGG, and KIPAN ($P < 0.05$). Among them, 27 were positive, and two were negative. Finally, in 32 cancers, including GBMLGG, KIPAN, and BLCA, RASSF1 expression was significantly correlated with the ESTIMATE score, with 29 significantly positive correlations and three significantly negative correlations ($P < 0.05$) (Supplementary Table 2). Fig. 7A shows the top three correlated cancers with sample numbers greater than 200.

Immune infiltrates have been linked to tumour progression and immunotherapy responses as major components of the TME. Therefore, we examined the correlation between RASSF1 expression in different immune cell types. Eight immune and two stromal cells were quantified using the MCP counter algorithm. We found that RASSF1 is associated with immune-infiltrating cells in the pancreas. In particular, in KIRP and KIPAN, RASSF1 had a significant moderate positive correlation with all eight immune cell types except T cells and neutrophils ($P < 0.05$). In particular, the expression of RASSF1 was positively correlated with T cells in 26 tumour types, CD8 + T cells in 30 tumour types, cytotoxic lymphocytes in 32 tumour types, B lineage in 20 tumour types, NK cells in 33 tumour types, monocytic lineage in 34 tumour types, myeloid dendritic cells in 28 tumour types, neutrophils in 29 tumour types, endothelial cells in 37 tumour types, and fibroblasts in 36 tumour types ($P < 0.05$) (Fig. 7B).

3.5. *Relationship between RASSF1 and RNA modification*

Recent studies have shown that RNA modifications, including m1A, m5C, and m6A, are important epigenetic modifications that participate in the regulation of tumour immune escape [30]. Therefore, we examined the relationship between RASSF1 expression and RNA modification in multiple cancers. The expression data of three RNA modification genes (10 m1A, 13 m5C, and 21 m6A) were extracted and correlation analysis was performed. The results showed that RASSF1 was associated with most of the RNA modification genes in multiple cancers. In ACC, LIHC, KIPAN, WT, and THCA, RASSF1 was associated with almost all RAN modifier genes ($P < 0.05$). Among them, YTHDC1 and YTHDF2, m1A readers; NSUN4, m5C authors; and METTL3, RBM15B, RBM15, and WTAP, m6A writers were positively correlated with RASSF1 in almost all tumours ($P < 0.05$) (Fig. 8).

3.6. *Role of RASSF1 in genomic heterogeneity*

Immunotherapy may be more beneficial for patients with high genomic instability because cancer is a genomic disease. To explore the role of RASSF1 in genomic heterogeneity, we calculated Spearman's correlations between RASSF1 and several genomic instability markers, including TMB, tumour neoantigen burden, MSI, tumour purity, and MATH, in each cancer type. We observed that RASSF1

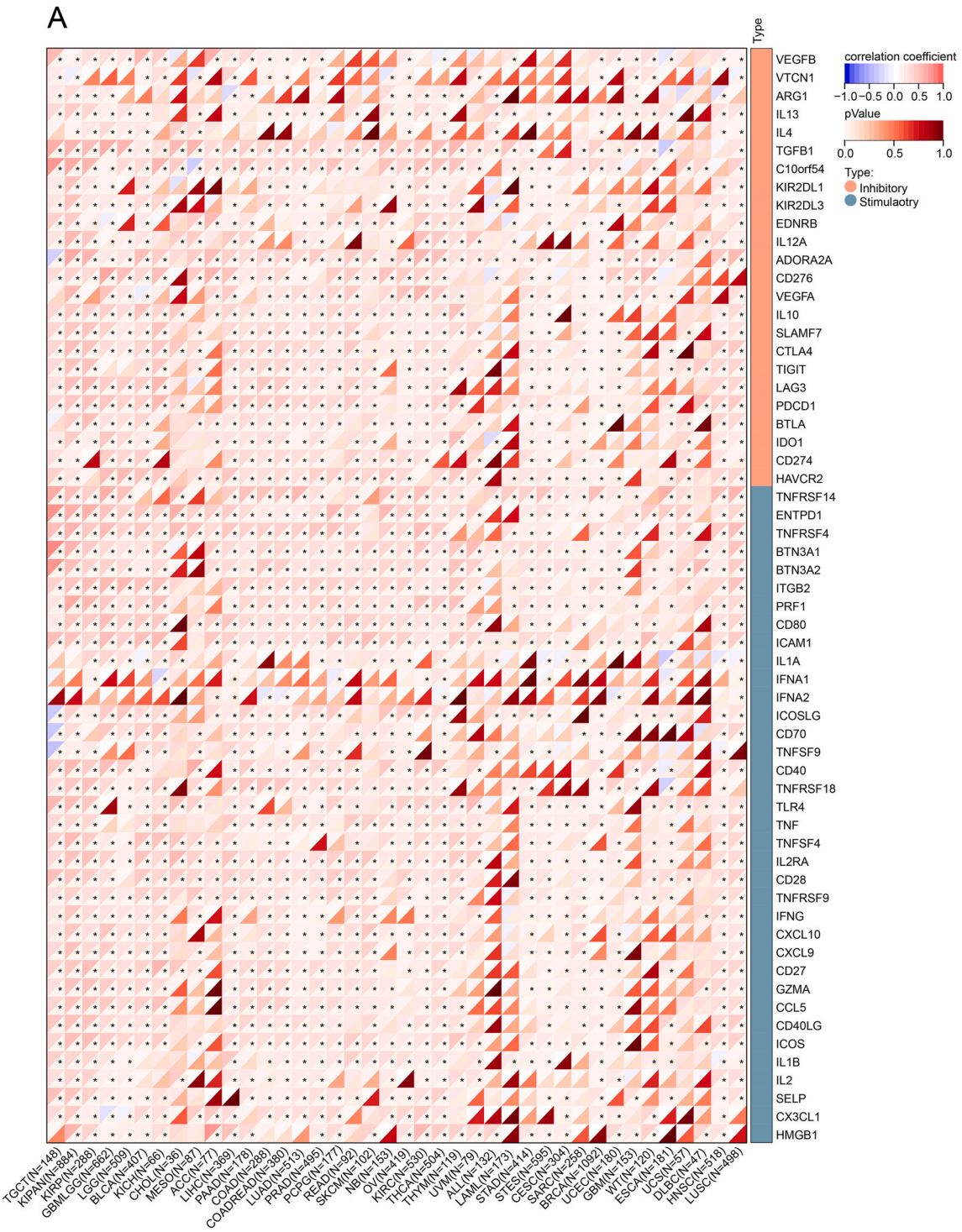


Fig. 6. RASSF1 is correlated with immune-related genes in pancancer. (A) Correlation analysis between RASSF1 expression and immune checkpoints. (B) Correlation analysis between RASSF1 expression and chemokines, chemokine receptors, and MHC molecules. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

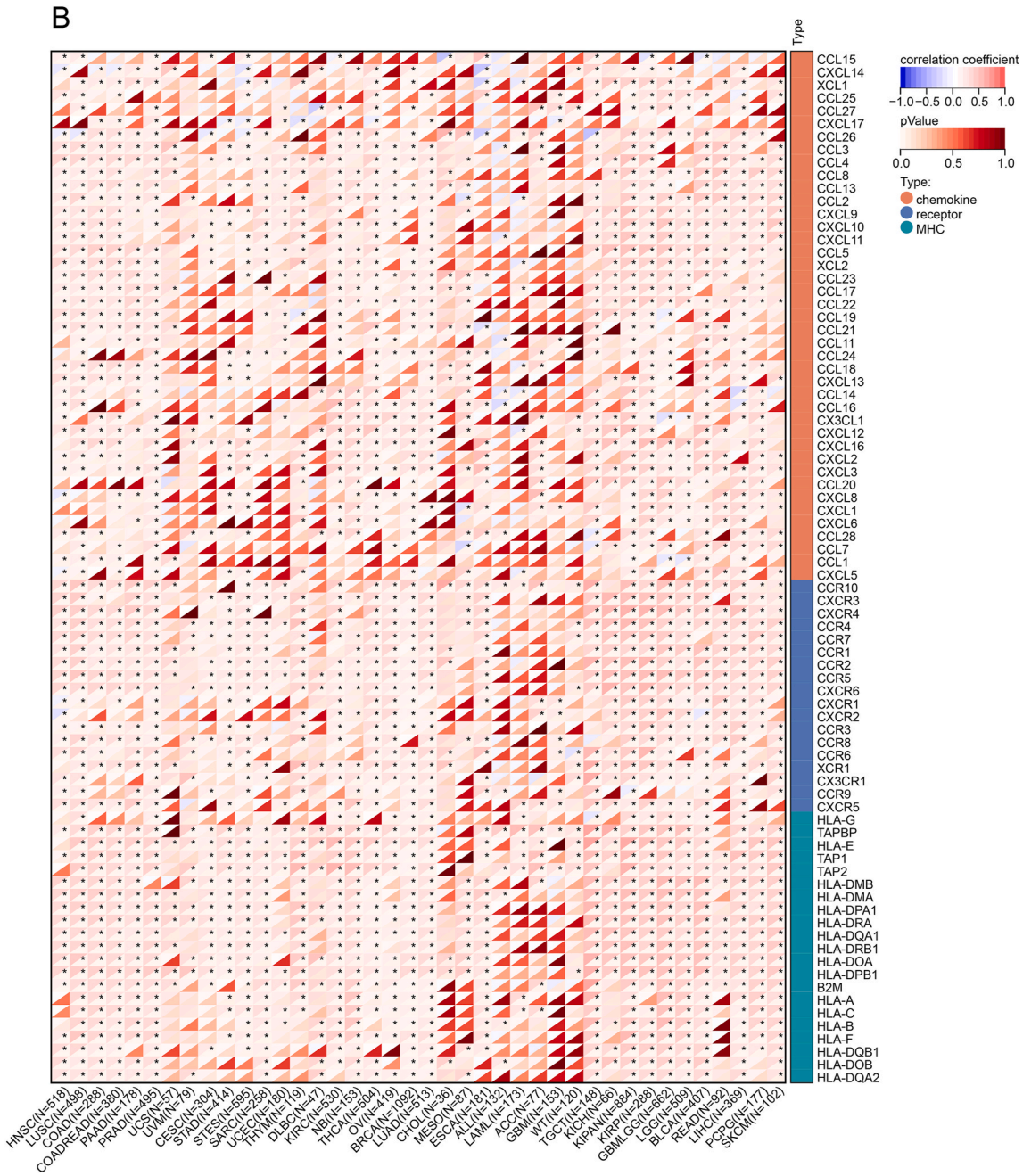


Fig. 6. (*continued*).

expression was significantly correlated with TMB in 16 cancers, among which RASSF1 was significantly positively correlated in 12 cancers, including GBMLGG, ACC, and ESCA, and significantly negatively correlated in four cancers, including DLBC, THCA, and BRCA (Fig. 9A). In addition, RASSF1 was significantly correlated with tumour neoantigens in five cancers, including a significant positive correlation in COAD, COADREAD, and UCEC, and a significant negative correlation in BRCA and HNSC (Fig. 9B). Regarding MSI, RASSF1 and MSI were significantly correlated in 16 cancers, including MESO, STAD, LUAD, and 13 other cancers, and they were significantly negatively correlated in CHOL, GBMLGG, and KIPAN (Fig. 9C). Furthermore, we observed a significant correlation between RASSF1 and tumour purity in 17 cancers, including a significant positive correlation in GBM and a significant negative correlation in 16 cancers, including KIPAN, BLCA, and PCPG (Fig. 9D). Finally, RASSF1 and MATH were significantly correlated in 11 cancers, among which there was a significant positive correlation in UVM and a significant negative correlation in 10 cancers, including TGCT, GBMLGG, and UCEC (Fig. 9E).

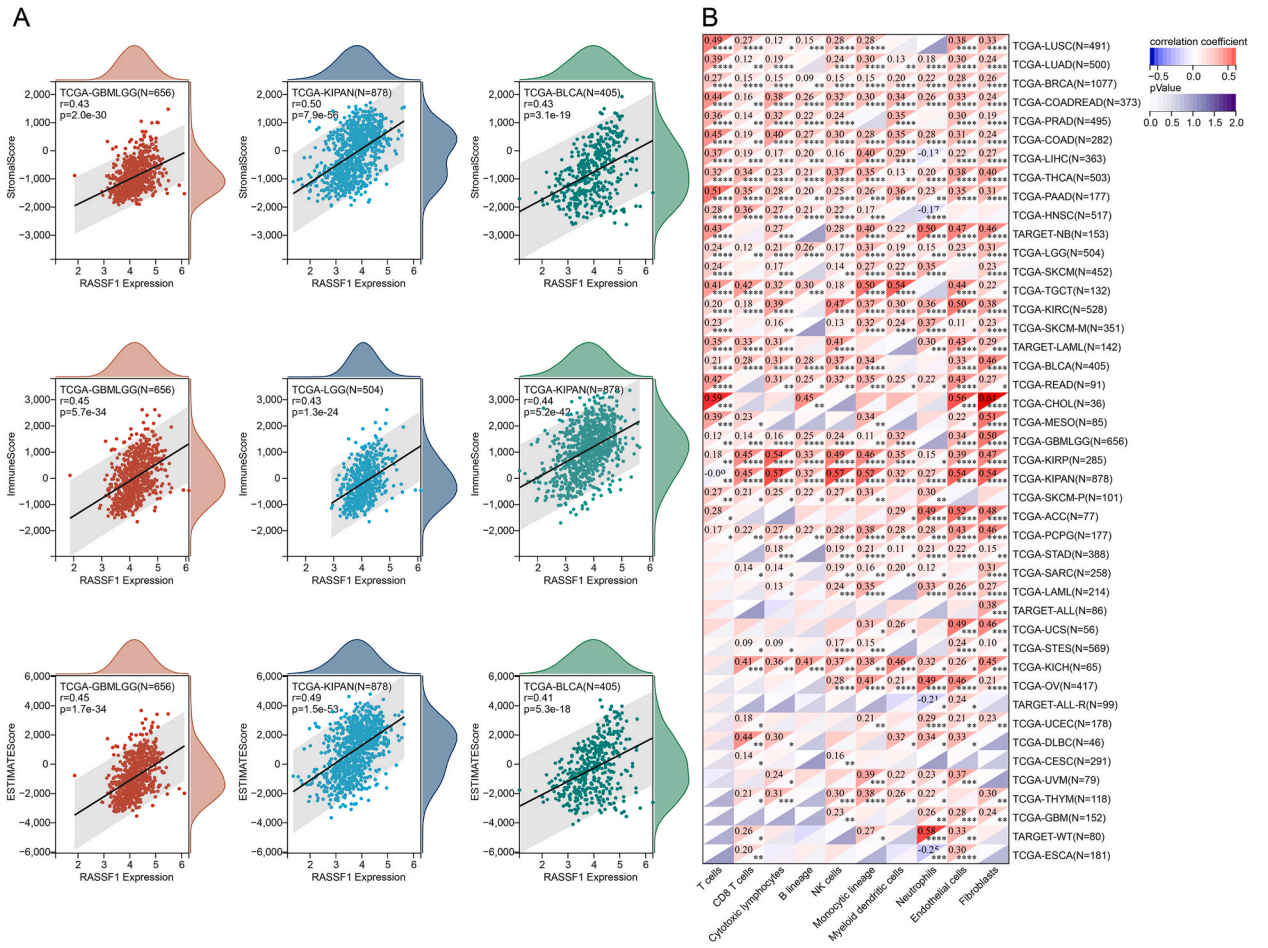


Fig. 7. RASSF1 affects the tumor microenvironment and immune infiltration across tumours. (A) Association of RASSF1 expression with stromal, immune, and ESTIMATE scores. (B) Association of RASSF1 expression with T cells, CD8 T cells, cytotoxic lymphocytes, B lineage cells, NK cells, monocyte lineage cells, myeloid dendritic cells, neutrophils, endothelial cells, and fibroblasts. *P < 0.05.

3.7. Correlation between RASSF1 expression and tumour stemness

Tumour stemness, closely related to immune escape, plays a crucial role in tumorigenesis and resistance to immunotherapy. To explore the role of RASSF1 in tumour stemness, we calculated Spearman's correlation between RASSF1 and the tumour stemness index (DNAsi and mRNAsi) in each tumour type. RASSF1 significantly correlated with DNAsi expression in 13 tumours. Among them, there was a significant positive correlation in seven tumours, including GBMLGG, MESO, and LGG, and a significant negative correlation in six tumours, including TGCT, BLCA, and LAML. Additionally, RASSF1 and mRNAsi were significantly correlated in 21 tumours, including positive correlations in STES and STAD, and negative correlations in 19 tumours, including TGCT, KIPAN, and KIRP (Fig. 10).

3.8. 8.RASSF1 is involved in the regulation of multiple immune-related signaling pathways

To elucidate the underlying mechanisms, we first performed PPI network analysis to reveal the functional network of RASSF1. The results demonstrated that RASSF1 is linked to RASSF5, KRAS, NRAS, HRAS, DAXX, XPA, STK3, SAV1, STK4, and MAP1S (Fig. 11A). GO enrichment analysis showed that RASSF1 is involved in multiple immune-related biological processes, such as the immune response-regulating signalling pathway, lymphocyte differentiation, and positive regulation of cytokine production (Fig. 11B). In addition, RASSF1 was enriched in immune-related KEGG signalling pathways, including the chemokine signalling pathway, T-cell receptor signalling pathway, and leukocyte transendothelial migration (Fig. 11C).

3.9. RASSF1A correlated with PD-L1 expression and affected the expression of Th17 signaling pathway genes in lung cancer

According to the above analysis, RASSF1 expression was significantly lower in NSCLC tissues than in normal lung tissues, and

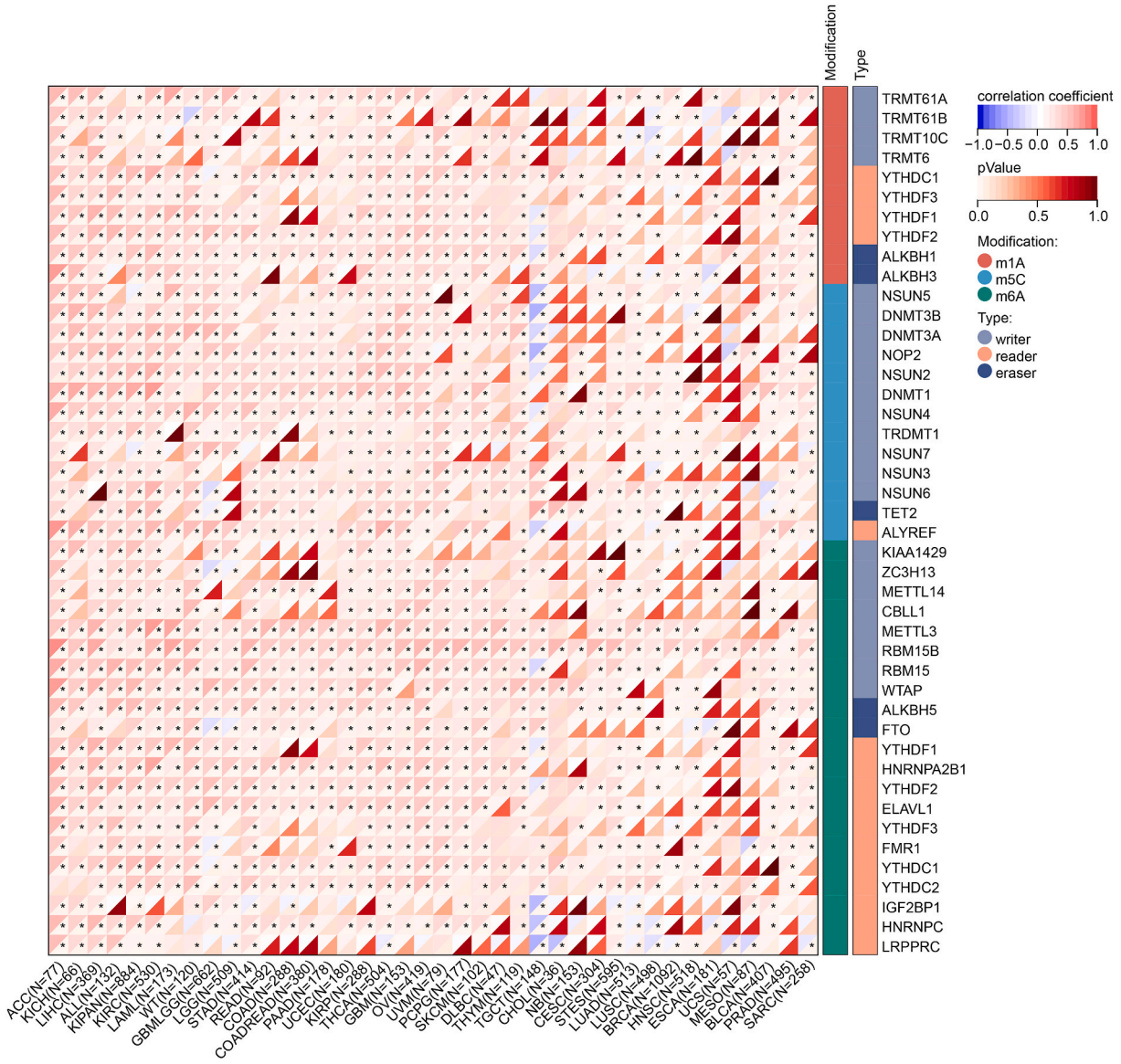
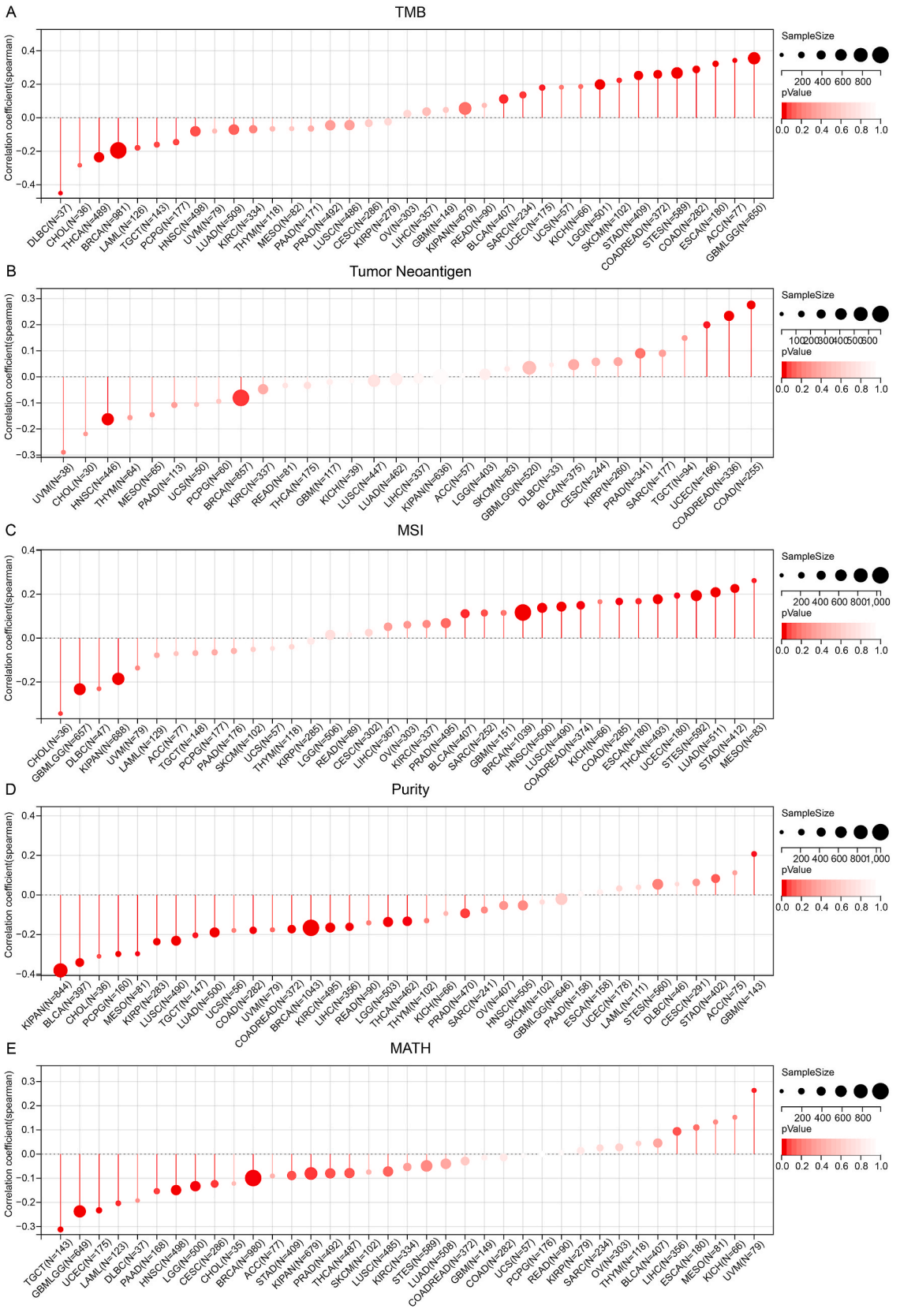


Fig. 8. Relationship between RASSF1 and RNA modification. *P < 0.05.

RASSF1 expression was significantly correlated with PD-L1 expression in NSCLC. Therefore, we examined the Human Protein Atlas (HPA) database for IHC staining of normal lung and NSCLC tissues, which showed that NSCLC tissues expressed lower levels of RASSF1 than normal lung tissues (Fig. 12A). In addition, we detected the expression of RASSF1A and PD-L1 in 11 NSCLC samples. Two typical examples are shown in Fig. 12B: one was negative for both RASSF1A and PD-L1 expression, and the other was positive for both RASSF1A and PD-L1 expression. Spearman's correlation analysis of 11 NSCLC samples revealed a moderate correlation between RASSF1A and PD-L1 expression (R = 0.556), but the difference was not statistically significant (P = 0.076, Fig. 12C). Previous studies have shown that Th17 cell differentiation is inhibited by the upregulation of PD-L1 [31]. In our study, RASSF1 was significantly enriched in the Th17 cell differentiation signaling pathway. Therefore, we constructed a RASSF1A overexpression A549 cell line. Primers for Th17 cell differentiation pathway-related genes were designed, and qPCR experiments were performed. These results suggested that the expression of several genes in the Th17 cell differentiation signaling pathway was significantly affected by high RASSF1A expression. The mRNA expression levels of CCL2, FOSB, MAPK4, MUC5B, NFKB1, S100A8, S100A9, TNF, and TNFA2P3 were significantly decreased in the RASSF1A overexpression A549 cell line compared to the control group, whereas the mRNA expression levels of CSF3, CXL3, FOSL1, and IKBKE were significantly higher (P < 0.05) (Fig. 12D).



(caption on next page)

Fig. 9. Role of RASSF1 in genomic heterogeneity. (A) Correlation analysis between RASSF1 expression and TMB. (B) Correlation analysis between RASSF1 expression and tumor neoantigens. (C) Correlation analysis between RASSF1 expression and MSI. (D) Correlation analysis between RASSF1 expression and purity. (E) Correlation analysis between RASSF1 expression and MATH. TMB, tumor mutation burden; MSI, microsatellite instability; MATH, mutant-allele tumor heterogeneity.

4. Discussion

Our study revealed that RASSF1 expression was significantly lower in tumour tissues of BRCA, LUAD, LUSC, and 20 other types of cancers compared to normal tissues, which is consistent with previous research indicating that RASSF1 is generally inactivated in malignant tumours [32]. However, RASSF1 expression was also higher in nine tumour types, including CHOL, GBM, and HNSC, providing new insights into the pan-cancer expression of RASSF1. In other words, the state of RASSF1 expression differed among different tumour types. Additionally, our study found that RASSF1 has prognostic value in a variety of tumours. Cox proportional hazards regression analysis revealed that the OS of patients with high RASSF1 expression in GBMLGG, LGG, LAML, and nine other tumour types was poor. Only three tumours (HNSC, TARGET-NB, and TARGET-ALL-R) showed that low expression of RASSF1 was associated with poor OS. Similar results were obtained using Kaplan–Meier survival analysis. In seven cancers, including ACC, BLCA, and GBM, high RASSF1 expression was associated with poor OS, whereas in HNSC and THYM, high RASSF1 expression correlated with good OS. Furthermore, the differential expression of RASSF1 also affected DSS, PFI and DFI in multiple tumours, but whether RASSF1A or RASSF1C has an effect needs to be further verified.

Immunotherapy is transforming the treatment of cancer like never before, allowing a substantial number of patients to achieve sustained cancer control. Most notably, a growing number of solid and haematologic cancers have been shown to respond well to immune checkpoint inhibitors (ICIs) that reactivate dysfunctional and/or exhausted T cells [33,34]. Therefore, we investigated the role of RASSF1 in regulating immune checkpoints. The results revealed that RASSF1 positively correlated with immune checkpoint molecules in most tumour types. In TGCT, KIPAN, KIRP, GBMLGG, LGG, and BLCA, RASSF1 expression correlated with almost all immune checkpoint molecules. Currently, PD-L1 and CTLA4 are the main targets of immunotherapy. We suggest that RASSF1 has a significant positive correlation with PD-L1 in 24 tumour types and with CTLA4 in 31 tumour types. In addition, our study revealed the co-expression of RASSF1 with genes encoding chemokines, chemokine receptors, and MHC molecules. These results indicate that RASSF1 expression is closely related to tumour immunity and suggest a new target for the development of immune activator agents.

Cancer is a complex ecosystem composed of tumour cells and a large number of non-tumour cells embedded in an aberrant

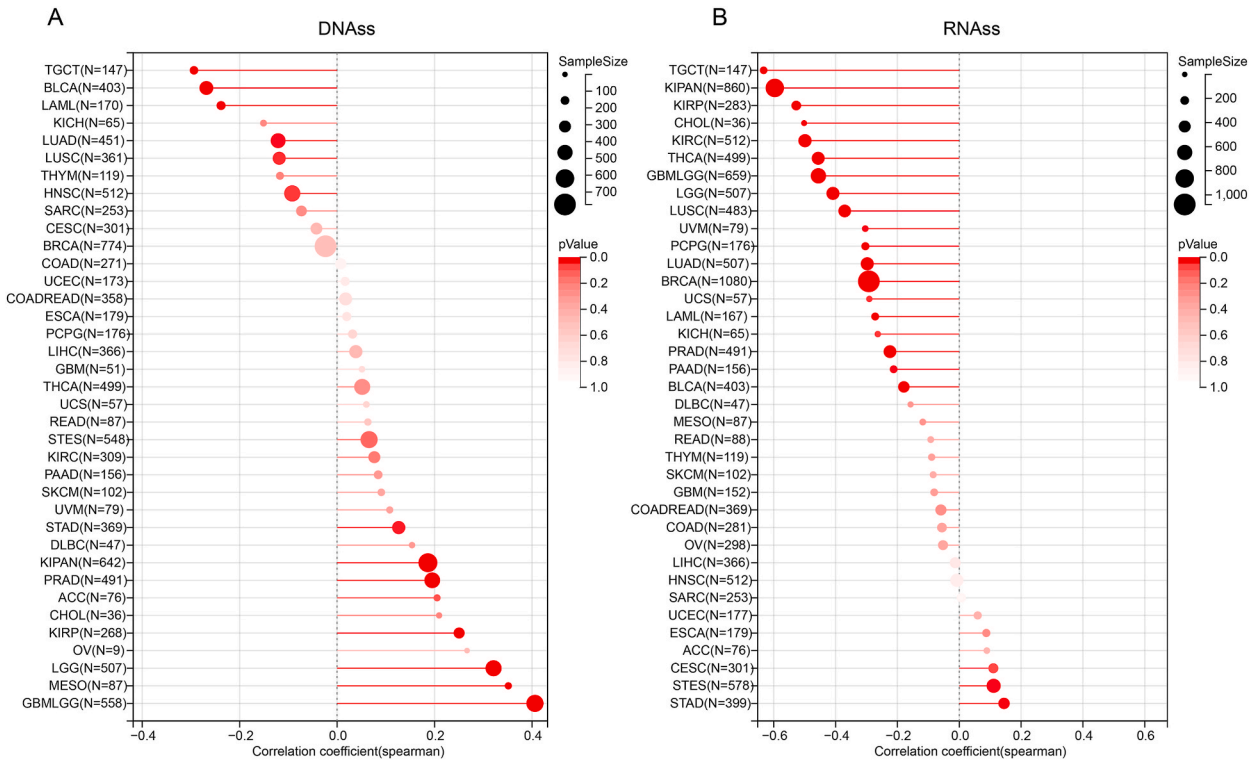


Fig. 10. Correlation between RASSF1 expression and tumor stemness. (A) Correlation analysis between RASSF1 expression and DNAsi. (B) Correlation analysis between RASSF1 expression and mRNAsi. DNAsi, DNA methylation-based stemness index; mRNAsi, mRNA expression-based stemness index.

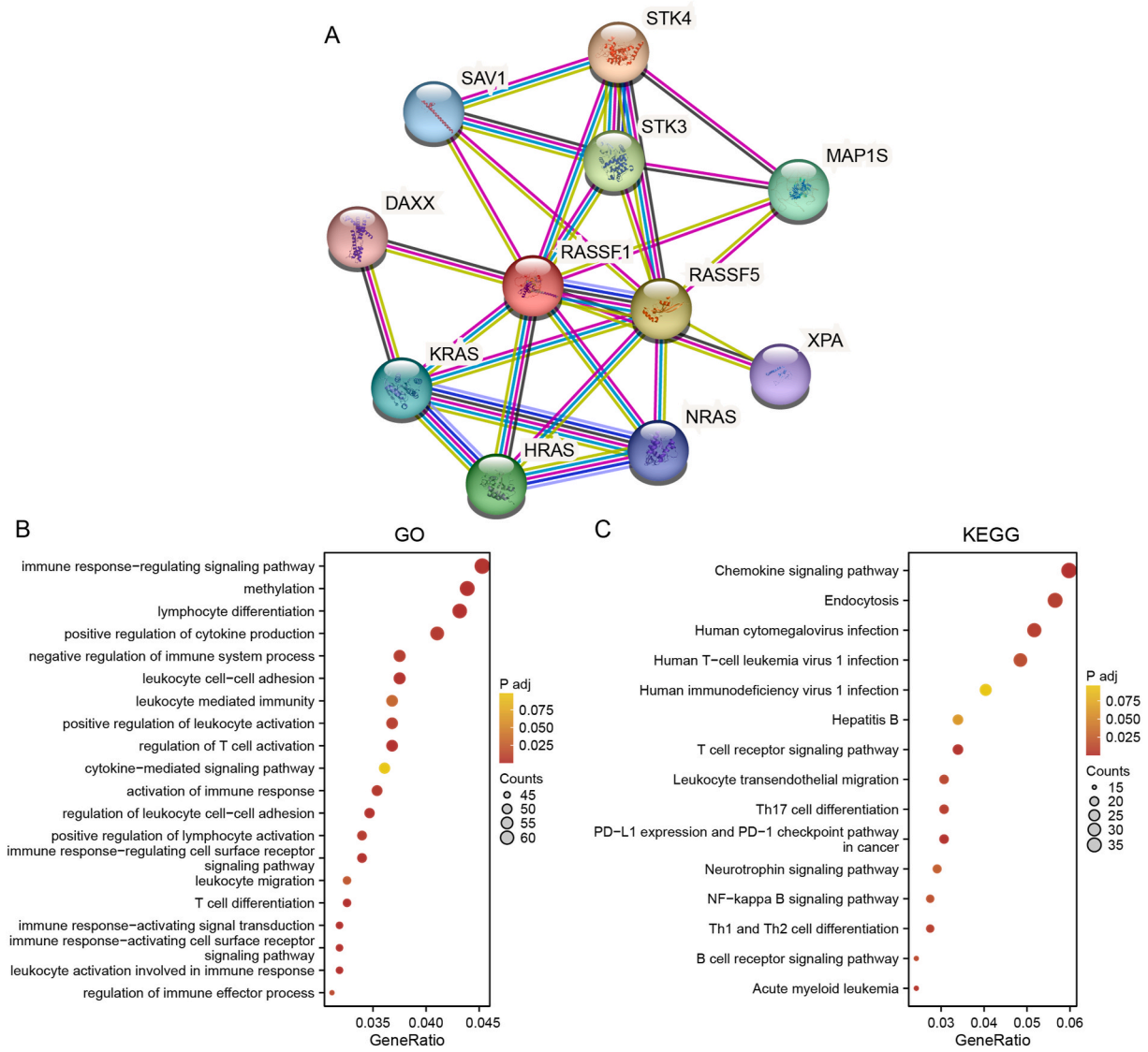


Fig. 11. RASSF1 is involved in the regulation of multiple immune-related signaling pathways. (A) PPI network analysis of RASSF1. (B) GO enrichment analysis of RASSF1. (C) KEGG enrichment analysis of RASSF1. PPI, Protein−Protein Interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopaedia of Genes and Genomes.

extracellular matrix. In addition to affecting cancer occurrence and development, the TME profoundly affects antitumour immunotherapy efficacy [35]. We found that RASSF1 expression significantly correlated with stromal, immune, and ESTIMATE scores in most cancers. Furthermore, we analysed the correlation between the expression of RASSF1 and immune infiltrating cells, which are major components of the TME. The results showed that RASSF1 positively correlated with infiltrating immune cells in all cancer types. In KIRP and KIPAN, RASSF1 was moderately correlated with all immune cells, except T cells and neutrophils. Schmidt et al. constructed a transgenic mouse model for the activation of K-RAS and defectiveness of RASSF1A in the lungs and found that inflammation and IL-6 production in RASSF1A-deficient tumours were significantly increased [36]. In addition, Zhang et al. found that the inhibition of RASSF1A promotes B-cell transformation [20]. Therefore, further investigation of the role of RASSF1 in inflammation and tumour immunity is required.

Immunosuppression is a representative feature of the TME. Although ICIs are stimulating huge waves in the field of tumour immune therapy, primary and secondary resistance have restricted their clinical application. Recent clinical trials have reported that epigenetic agents can significantly improve by epigenetic therapy, indicating the importance of epigenetic modifications in tumour immune regulation. In recent years, RNA modifications (N1-methyladenosine [m1A], 5-methylcytosine [m5C], and N6-methyladenosine [m6A]) have become new hotspots for antitumour immunity [30]. Our results showed that RASSF1 was associated with most RNA modification genes in multiple cancers. Especially with almost all RAN modifier genes, particularly ACC, LIHC, KIPAN, WT, and THCA RASSF1 was associated with almost all RAN modifier genes. As the most abundant RNA epigenetic modification, m6A regulates

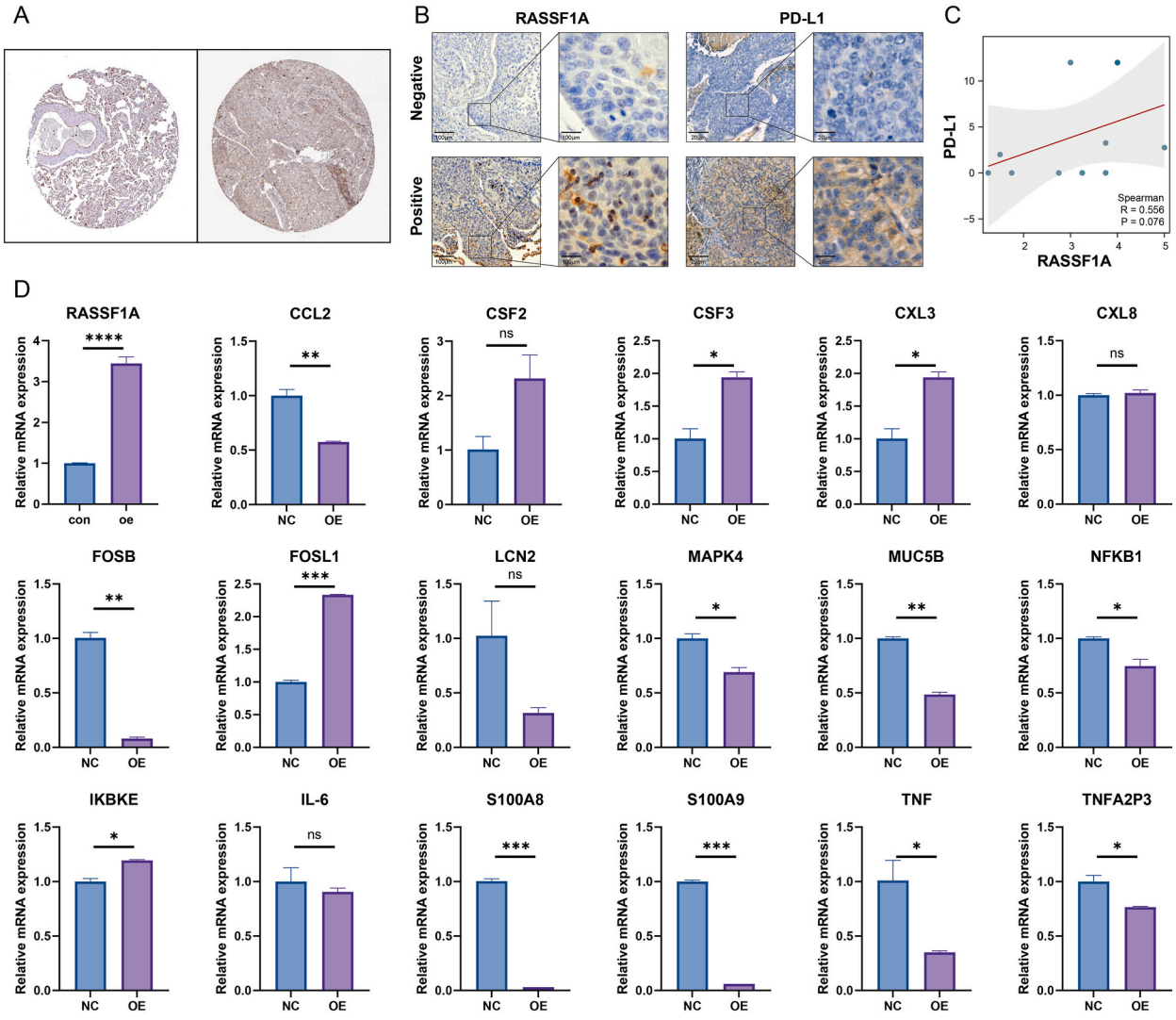


Fig. 12. RASSF1A correlated with PD-L1 expression and affected the expression of Th17 signaling pathway genes in lung cancer. (A) Representative immunohistochemical staining for RASSF1 in A normal lung tissue and B NSCLC. (B) Two typical examples of RASSF1A and PD-L1 expression. (C) Spearman's correlation analysis between RASSF1A and PD-L1 expression in 11 NSCLC samples. (D) mRNA expression of RASSF1A, CCL2, CSF2, CSF3, CXL3, CXL8, FOSB, FOSL1, LCN2, MAPK4, MUC5B, NFKB1, IKBKE, IL-6, S100A8, S100A9, TNF, and TNFA2P3 in negative control and RASSF1A overexpression A549 cells.

tumour immunogenicity and affects antitumour responses [37]. Our analysis revealed that the m6A writers METTL3, RBM15B, RBM15, and WTAP positively correlated with RASSF1 in almost all tumour types. It has been suggested that METTL3 can positively regulate the survival of natural killer (NK) cells, promote the production of proinflammatory cytokines in macrophages, and promote the dynamic balance and differentiation of CD4+T cells [37]. These results suggest that RASSF1 alters the immune status of the TME by regulating the expression of METTL3 in multiple tumours. Therefore, it is necessary to examine the role of RASSF1 in RNA modification to identify new targets for immunotherapy.

Tumour heterogeneity is driven by the continuous generation of new genetic variants that promote tumour progression and treatment resistance. Tumours with unstable genomes generally have a poor prognosis under traditional treatment modalities. In contrast, genomic instability triggers downstream inflammatory signals that can enhance antitumour immunity and sensitisation to ICIs therapy [29,38]. Genomic heterogeneity was evaluated in various ways, including TMB, tumour neoantigens, MSI, purity, and MATH. TMB has been shown to be a useful biomarker for ICI selection in several cancer types [39]. Tumour neoantigens are a new area of cancer immunotherapy, and many clinical trials have validated neoantigen-based therapies such as cancer vaccines [40]. In 2017, the Food and Drug Administration (FDA) approved the PD-1 inhibitor pembrolizumab for the treatment of MSI-H/dMMR solid tumours, becoming the world's first antitumour therapy based on biomarkers rather than tumour origin [41]. Our study showed that RASSF1 was significantly associated with TMB in 16 tumours, tumour neoantigens in five tumours, and MSI in 16 tumours. Our results

suggest that RASSF1 is closely associated with genomic instability. Therefore, RASSF1 may serve as a new marker for predicting the efficacy of immunotherapy.

Cancer progression involves the progressive loss of differentiation phenotypes and the acquisition of progenitor features. Tumour stemness is closely related to immune escape and plays a crucial role in tumour occurrence and resistance to immunotherapy resistance [29,42]. DNAsi calculated by the methylation signature and mRNAsi calculated by the mRNA signature were used as tumour stemness scores. We observed a significant association between RASSF1 and DNAsi in 13 tumours and between RASSF1 and mRNAsi in 21 tumours. Consistent with this, Pankova et al. identified RASSF1A as a clinical biomarker associated with the mechanical properties of the extracellular matrix, increasing tumour stemness and the risk of metastatic progression in lung adenocarcinoma [43]. However, no studies have explored the correlation between the effect of RASSF1 on tumour cell stemness and tumour immunotherapy resistance, which may be a promising research direction.

Moreover, PPI network analysis showed that RASSF1 was linked to RASSF5, KRAS, NRAS, HRAS, DAXX, XPA, STK3, SAV1, STK4, and MAP1S. Consistent with previous studies, RASSF1 mainly interacted with RAS and Hippo pathway-related proteins. Further GO enrichment analysis revealed that RASSF1 is involved in multiple immune-related biological processes such as lymphocyte differentiation, immune response-regulating signaling pathways, and positive regulation of cytokine production. Additionally, RASSF1 is implicated in immune-related KEGG signaling pathways, including the chemokine signaling pathway, T-cell receptor signaling pathway, and leukocyte transendothelial migration. Finally, IHC staining and qPCR were performed to verify the previous findings. We found that RASSF1A was moderately correlated with PD-L1, and that RASSF1A overexpression significantly affected the expression of several genes in the Th17 cell differentiation signaling pathway. Previous studies have shown that PD-L1 upregulation inhibits Th17 cell differentiation [31]. We hypothesized that RASSF1A affects Th17 cell differentiation by upregulating PD-L1; however, further experiments are needed to validate this assumption.

Our RASSF1 pan-cancer study covers various types of cancer and helps to reveal common features and differences between different cancers, thereby providing comprehensive information for cancer diagnosis, treatment, and prevention. In addition, our study is the first to explore the role of RASSF1 in tumour immunity, and the results suggest that RASSF1 may be an emerging immune-related biomarker that plays an important regulatory role in tumour immunity. However, our study has some limitations. We only performed bioinformatics analysis on an open and accessible database and performed a simple validation in lung cancer. However, the role of RASSF1 in tumour immunity is extremely complex, and further clinical studies with larger sample sizes and more in-depth basic research are required to verify our findings.

5. Conclusion

In summary, we found that the expression of RASSF1 was altered in multiple tumours compared to that in adjacent tissues and was significantly correlated with prognosis, suggesting that RASSF1 is a potential prognostic biomarker. In addition, RASSF1 is correlated with immune checkpoint molecules, the tumour immune microenvironment, RNA modification, and tumour stemness. GO and KEGG enrichment analyses suggested that RASSF1 is closely associated with a variety of immune-related pathways. This suggests that RASSF1 plays an important role in tumour immunity. Next, we validated some of these findings in lung cancer and found that RASSF1A correlated with PD-L1 expression and that RASSF1A overexpression significantly affected the expression of multiple genes in the Th17 cell differentiation signalling pathway in lung cancer. These findings may elucidate the value of RASSF1 in tumour development and prognosis and open new avenues for individualised immunotherapy.

Funding

None.

Declarations

Ethics approval and consent to participate

Immunohistochemical specimens were reviewed and approved by the Ethics Committee of Chinese PLA General Hospital (S2021-553-01, October 9, 2021). All patients provided written informed consent, in accordance with the principles of the Declaration of Helsinki.

Consent for publication

Not applicable.

Data availability statement

The datasets analysed in this study are available from the Cancer Genome Atlas (https://cancergenome.nih.gov/), CCLE (https://portals.broadinstitute.org/ccle/), GTEx (https://commonfund.nih.gov/GTEx/), and Human Protein Atlas (https://www.proteinatlas.org/).

CRediT authorship contribution statement

Yibing Bai: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yuanyong Wang:** Visualization, Software, Investigation, Formal analysis, Data curation. **Jiapei Qin:** Visualization, Validation, Investigation, Formal analysis. **Ting Wang:** Writing – original draft, Visualization, Software, Investigation. **Xin Zhou:** Investigation. **Zhiqiang Ma:** Investigation. **An Wang:** Investigation. **Wenyu Yang:** Investigation. **Jinliang Wang:** Writing – review & editing. **Jinfeng Li:** Writing – review & editing. **Yi Hu:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We acknowledge our use of the R software. The results are in part based on data derived from TCGA, CCLE, GTE, TCGA, TARGET, and HPA databases. We appreciate the platforms and authors who uploaded the data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33304.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA A Cancer J. Clin. 68 (2018) 394–424, https://doi.org/10.3322/caac.21492.

[2] E.B. Garon, M.D. Hellmann, N.A. Rizvi, E. Carcereny, N.B. Leighl, M.J. Ahn, J.P. Eder, A.S. Balmanoukian, C. Aggarwal, L. Horn, A. Patnaik, M. Gubens, S. S. Ramalingam, E. Felip, J.W. Goldman, C. Scalzo, E. Jensen, D.A. Kush, R. Hui, Five-year overall survival for patients with advanced non–small-cell lung cancer treated with pembrolizumab: results from the phase I KEYNOTE-001 study, J. Clin. Oncol. : official journal of the American Society of Clinical Oncology 37 (2019) 2518–2527, https://doi.org/10.1200/jco.19.00934.

[3] P.M. Forde, J.E. Chaft, K.N. Smith, V. Anagnostou, T.R. Cottrell, M.D. Hellmann, M. Zahurak, S.C. Yang, D.R. Jones, S. Broderick, R.J. Battafarano, M.J. Velez, N. Rekhtman, Z. Olah, J. Naidoo, K.A. Marrone, F. Verde, H. Guo, J. Zhang, J.X. Caushi, H.Y. Chan, J.W. Sidhom, R.B. Scharpf, J. White, E. Gabrielson, H. Wang, G.L. Rosner, V. Rusch, J.D. Wolchok, T. Merghoub, J.M. Taube, V.E. Velculescu, S.L. Topalian, J.R. Brahmer, D.M. Pardoll, Neoadjuvant PD-1 blockade in resectable lung cancer, N. Engl. J. Med. 378 (2018) 1976–1986, https://doi.org/10.1056/NEJMoa1716078.

[4] G. Malpeli, G. Innamorati, I. Decimo, M. Bencivenga, A.H. Nwabo Kamdje, R. Perris, C. Bassi, Methylation dynamics of RASSF1A and its impact on cancer, Cancers 11 (2019), https://doi.org/10.3390/cancers11070959.

[5] A.M. Grawenda, E. O'Neill, Clinical utility of RASSF1A methylation in human malignancies, Br. J. Cancer 113 (2015) 372–381, https://doi.org/10.1038/bjc.2015.221.

[6] H. Gao, J. Yang, L. He, W. Wang, Y. Liu, Y. Hu, M. Ge, J. Ding, Q. Ye, The diagnostic potential of SHOX2 and RASSF1A DNA methylation in early lung adenocarcinoma, Frontiers in oncology 12 (2022) 849024, https://doi.org/10.3389/fonc.2022.849024.

[7] C. Zhang, W. Yu, L. Wang, M. Zhao, Q. Guo, S. Lv, X. Hu, J. Lou, DNA methylation analysis of the SHOX2 and RASSF1A panel in bronchoalveolar lavage fluid for lung cancer diagnosis, J. Cancer 8 (2017) 3585–3591, https://doi.org/10.7150/jca.21368.

[8] M. Li, C. Wang, B. Yu, X. Zhang, F. Shi, X. Liu, Diagnostic value of RASSF1A methylation for breast cancer: a meta-analysis, Biosci. Rep. 39 (2019), https://doi.org/10.1042/bsr20190923.

[9] S. Salta, P.N. S, M. Fontes-Sousa, P. Lopes, M. Freitas, M. Caldas, L. Antunes, F. Castro, P. Antunes, S. Palma de Sousa, R. Henrique, C. Jerónimo, A DNA methylation-based test for breast cancer detection in circulating cell-free DNA, J. Clin. Med. 7 (2018), https://doi.org/10.3390/jcm7110420.

[10] J.H. Yoon, R. Dammann, G.P. Pfeifer, Hypermethylation of the CpG island of the RASSF1A gene in ovarian and renal cell carcinomas, Int. J. Cancer 94 (2001) 212–217, https://doi.org/10.1002/ijc.1466.

[11] F. Hu, L. Chen, M.Y. Bi, L. Zheng, J.X. He, Y.Z. Huang, Y. Zhang, X.L. Zhang, Q. Guo, Y. Luo, W.R. Tang, M.M. Sheng, Potential of RASSF1A promoter methylation as a biomarker for colorectal cancer: meta-analysis and TCGA analysis, Pathol. Res. Pract. 216 (2020) 153009, https://doi.org/10.1016/j.prp.2020.153009.

[12] A.K. Kim, J.P. Hamilton, S.Y. Lin, T.T. Chang, H.W. Hann, C.T. Hu, Y. Lou, Y.J. Lin, T.P. Gade, G. Park, H. Luu, T.J. Lee, J. Wang, D. Chen, M.G. Goggins, S. Jain, W. Song, Y.H. Su, Urine DNA biomarkers for hepatocellular carcinoma screening, Br. J. Cancer 126 (2022) 1432–1438, https://doi.org/10.1038/s41416-022-01706-9.

[13] S.M. Khoshfetrat, P. Seyed Dorraji, M. Shayan, F. Khatami, K. Omidfar, Smartphone-based electrochemiluminescence for visual simultaneous detection of RASSF1A and SLC5A8 tumor suppressor gene methylation in thyroid cancer patient plasma, Analytical chemistry 94 (2022) 8005–8013, https://doi.org/10.1021/acs.analchem.2c01132.

[14] H. Zhang, S. Zhang, Z. Zhang, H. Jia, S. Gu, D. Zhao, [Prognostic value of methylation status of RASSF1A gene as an independent factor of non-small cell lung cancer], Zhongguo fei ai za zhi = Chinese journal of lung cancer 13 (2010) 311–316, https://doi.org/10.3779/j.issn.1009-3419.2010.04.08.

[15] C. Feng, J. Dong, W. Chang, M. Cui, T. Xu, The progress of methylation regulation in gene expression of cervical cancer, Int J Genomics 2018 (2018) 8260652, https://doi.org/10.1155/2018/8260652.

[16] Q. Guo, H.B. Wang, Y.H. Li, H.F. Li, T.T. Li, W.X. Zhang, S.S. Xiang, Z.Q. Sun, Correlations of promoter methylation in WIF-1, RASSF1A, and CDH13 genes with the risk and prognosis of esophageal cancer, Med. Sci. Mon. Int. Med. J. Exp. Clin. Res. 22 (2016) 2816–2824, https://doi.org/10.12659/msm.896877.

[17] L. García-Gutiérrez, S. McKenna, W. Kolch, D. Matallanas, RASSF1A tumour suppressor: target the network for effective cancer therapy, Cancers 12 (2020), https://doi.org/10.3390/cancers12010229.

[18] T. Moroishi, T. Hayashi, W.W. Pan, Y. Fujita, M.V. Holt, J. Qin, D.A. Carson, K.L. Guan, The Hippo pathway kinases LATS1/2 suppress cancer immunity, Cell 167 (2016) 1525–1539.e1517, https://doi.org/10.1016/j.cell.2016.11.005.
[19] A. Dey, X. Varelas, K.L. Guan, Targeting the Hippo pathway in cancer, fibrosis, wound healing and regenerative medicine, Nat. Rev. Drug Discov. 19 (2020) 480–494, https://doi.org/10.1038/s41573-020-0070-z.
[20] S. Zhang, Y. Pei, F. Lang, K. Sun, R.K. Singh, Z.L. Lamplugh, A. Saha, E.S. Robertson, EBNA3C facilitates RASSF1A downregulation through ubiquitin-mediated degradation and promoter hypermethylation to drive B-cell proliferation, PLoS Pathog. 15 (2019) e1007514, https://doi.org/10.1371/journal.ppat.1007514.
[21] J. Li, F. Wang, A. Protopopov, A. Malyukova, V. Kashuba, J.D. Minna, M.I. Lerman, G. Klein, E. Zabarovsky, Inactivation of RASSF1C during in vivo tumor growth identifies it as a tumor suppressor gene, Oncogene 23 (2004) 5941–5949, https://doi.org/10.1038/sj.onc.1207789.
[22] E. Estrabaud, I. Lassot, G. Blot, E. Le Rouzic, V. Tanchou, E. Quemeneur, L. Daviet, F. Margottin-Goguet, R. Benarous, RASSF1C, an isoform of the tumor suppressor RASSF1A, promotes the accumulation of beta-catenin by interacting with betaTrCP, Cancer Res. 67 (2007) 1054–1061, https://doi.org/10.1158/0008-5472.Can-06-2530.
[23] Y.G. Amaar, M.G. Minera, L.K. Hatran, D.D. Strong, S. Mohan, M.E. Reeves, Ras association domain family 1C protein stimulates human lung cancer cell proliferation, Am. J. Physiol. Lung Cell Mol. Physiol. 291 (2006) L1185–L1190, https://doi.org/10.1152/ajplung.00072.2006.
[24] M.L. Tognoli, N. Vlahov, S. Steenbeek, A.M. Grawenda, M. Eyres, D. Cano-Rodriguez, S. Scrace, C. Kartsonaki, A. von Kriegsheim, E. Willms, M.J. Wood, M. G. Rots, J. van Rheenen, E. O'Neill, D. Pankova, RASSF1C oncogene elicits amoeboid invasion, cancer stemness, and extracellular vesicle release via a SRC/Rho axis, The EMBO journal 40 (2021) e107680, https://doi.org/10.15252/embj.2021107680.
[25] D. Zeng, Z. Ye, R. Shen, G. Yu, J. Wu, Y. Xiong, R. Zhou, W. Qiu, N. Huang, L. Sun, X. Li, J. Bin, Y. Liao, M. Shi, W. Liao, IOBR: multi-omics immuno-oncology biological research to decode tumor microenvironment and signatures, Front. Immunol. 12 (2021) 687975, https://doi.org/10.3389/fimmu.2021.687975.
[26] Y. Huang, W. Zhang, Q. Li, Z. Wang, X. Yang, Identification of m6A/m5C/m1A-associated LncRNAs for prognostic assessment and immunotherapy in pancreatic cancer, Sci. Rep. 13 (2023) 3661, https://doi.org/10.1038/s41598-023-30865-9.
[27] V. Thorsson, D.L. Gibbs, S.D. Brown, D. Wolf, D.S. Bortone, T.H. Ou Yang, E. Porta-Pardo, G.F. Gao, C.L. Plaisier, J.A. Eddy, E. Ziv, A.C. Culhane, E.O. Paull, I.K. A. Sivakumar, A.J. Gentles, R. Malhotra, F. Farshidfar, A. Colaprico, J.S. Parker, L.E. Mose, N.S. Vo, J. Liu, Y. Liu, J. Rader, V. Dhankani, S.M. Reynolds, R. Bowlby, A. Califano, A.D. Cherniack, D. Anastassiou, D. Bedognetti, Y. Mokrab, A.M. Newman, A. Rao, K. Chen, A. Krasnitz, H. Hu, T.M. Malta, H. Noushmehr, C.S. Pedamallu, S. Bullman, A.I. Ojesina, A. Lamb, W. Zhou, H. Shen, T.K. Choueiri, J.N. Weinstein, J. Guinney, J. Saltz, R.A. Holt, C.S. Rabkin, A.J. Lazar, J.S. Serody, E.G. Demicco, M.L. Disis, B.G. Vincent, I. Shmulevich, The immune landscape of cancer, Immunity 48 (2018) 812–830.e814, https://doi.org/10.1016/j.immuni.2018.03.023.
[28] R. Bonneville, M.A. Krook, E.A. Kautto, J. Miya, M.R. Wing, H.Z. Chen, J.W. Reeser, L. Yu, S. Roychowdhury, Landscape of Microsatellite Instability across 39 Cancer Types, JCO precision oncology, 2017, https://doi.org/10.1200/po.17.00073, 2017.
[29] T.M. Malta, A. Sokolov, A.J. Gentles, T. Burzykowski, L. Poisson, J.N. Weinstein, B. Kamińska, J. Huelsken, L. Omberg, O. Gevaert, A. Colaprico, P. Czerwińska, S. Mazurek, L. Mishra, H. Heyn, A. Krasnitz, A.K. Godwin, A.J. Lazar, J.M. Stuart, K.A. Hoadley, P.W. Laird, H. Noushmehr, M. Wiznerowicz, Machine learning identifies stemness features associated with oncogenic dedifferentiation, Cell 173 (2018) 338–354.e315, https://doi.org/10.1016/j.cell.2018.03.034.
[30] Y. Kong, J. Yu, S. Ge, X. Fan, Novel insight into RNA modifications in tumor immunity: promising targets to prevent tumor immune escape, Innovation 4 (2023) 100452, https://doi.org/10.1016/j.xinn.2023.100452.
[31] Y. Zhang, C.A. Ma, M.G. Lawrence, T.J. Break, M.P. O'Connell, J.J. Lyons, D.B. López, J.S. Barber, Y. Zhao, D.L. Barber, A.F. Freeman, S.M. Holland, M. S. Lionakis, J.D. Milner, PD-L1 up-regulation restrains Th17 cell differentiation in STAT3 loss- and STAT1 gain-of-function patients, J. Exp. Med. 214 (2017) 2523–2533, https://doi.org/10.1084/jem.20161427.
[32] F. Dubois, E. Bergot, G. Zalcman, G. Levallet, RASSF1A, puppeteer of cellular homeostasis, fights tumorigenesis, and metastasis-an updated review, Cell Death Dis. 10 (2019) 928, https://doi.org/10.1038/s41419-019-2169-x.
[33] I. Mellman, G. Coukos, G. Dranoff, Cancer immunotherapy comes of age, Nature 480 (2011) 480–489, https://doi.org/10.1038/nature10673.
[34] S.L. Topalian, J.M. Taube, R.A. Anders, D.M. Pardoll, Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy, Nat. Rev. Cancer 16 (2016) 275–287, https://doi.org/10.1038/nrc.2016.36.
[35] J.M. Pitt, A. Marabelle, A. Eggermont, J.C. Soria, G. Kroemer, L. Zitvogel, Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy, Ann. Oncol. : official journal of the European Society for Medical Oncology 27 (2016) 1482–1492, https://doi.org/10.1093/annonc/mdw168.
[36] M.L. Schmidt, K.R. Hobbing, H. Donninger, G.J. Clark, RASSF1A deficiency enhances RAS-driven lung tumorigenesis, Cancer Res. 78 (2018) 2614–2623, https://doi.org/10.1158/0008-5472.Can-17-2466.
[37] H. Huang, H. Weng, J. Chen, m(6)A modification in coding and non-coding RNAs: roles and therapeutic implications in cancer, Cancer Cell 37 (2020) 270–288, https://doi.org/10.1016/j.ccell.2020.02.004.
[38] M. Chen, R. Linstra, M. van Vugt, Genomic instability, inflammatory signaling and response to cancer immunotherapy, Biochim. Biophys. Acta Rev. Canc 1877 (2022) 188661, https://doi.org/10.1016/j.bbcan.2021.188661.
[39] T.A. Chan, M. Yarchoan, E. Jaffee, C. Swanton, S.A. Quezada, A. Stenzinger, S. Peters, Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic, Ann. Oncol. : official journal of the European Society for Medical Oncology 30 (2019) 44–56, https://doi.org/10.1093/annonc/mdy495.
[40] X. Zhao, X. Pan, Y. Wang, Y. Zhang, Targeting neoantigens for cancer immunotherapy, Biomark. Res. 9 (2021) 61, https://doi.org/10.1186/s40364-021-00315-7.
[41] L. Marcus, S.J. Lemery, P. Keegan, R. Pazdur, FDA approval summary: pembrolizumab for the treatment of microsatellite instability-high solid tumors, Clin. Cancer Res. : an official journal of the American Association for Cancer Research 25 (2019) 3753–3758, https://doi.org/10.1158/1078-0432.Ccr-18-4070.
[42] L. Jia, Y. Wang, C.Y. Wang, circFAT1 promotes cancer stemness and immune evasion by promoting STAT3 activation, Adv. Sci. 8 (2021) 2003376, https://doi.org/10.1002/advs.202003376.
[43] D. Pankova, Y. Jiang, M. Chatzifrangkeskou, I. Vendrell, J. Buzzelli, A. Ryan, C. Brown, E. O'Neill, RASSF1A controls tissue stiffness and cancer stem-like cells in lung adenocarcinoma, The EMBO journal 38 (2019) e100532, https://doi.org/10.15252/embj.2018100532.